

Open Literature Review

1. **Chemical Names:** Clothianidin, Chlorantraniliprole
2. **PC Codes:** 044309, 090100
3. **CAS Nos.** 210880-92-5, 500008-45-7
4. **MRID:** None
5. **ECOTOX Record Number and Citation:**
Larson, J.L., C.T. Redmond, and D.A. Potter. 2013. Assessing Insecticide Hazard to Bumble Bees Foraging on Flowering Weeds in Treated Lawns. *PLoS ONE*, 8(6): e66375.
6. **Purpose of Review:** Thiamethoxam and clothianidin re-evaluation for pollinators
7. **Date of Review:** 1/9/2015
8. **Description of Use:** Qualitative
9. **Summary of Study Findings:**

Executive Summary

This study evaluated bumble bee colony exposure and subsequent response to residues of clothianidin and chlorantraniliprole following foliar spray applications to flowering weeds in a treated lawn containing flowering white clover. Clothianidin residues on nectar from clover blooms after clothianidin spray application measured 171 ± 44 ppb a.i.. Residues of chlorantraniliprole were not able to be determined due to insufficient nectar collection, since the authors reported that these treated flowers as well as the untreated flowers had generally been pollinated. Colonies were confined to the treated and control areas to forage for six days and then moved to a non-treated site to openly forage and develop. Colonies exposed to the clothianidin-treated turf showed significantly reduced numbers of foragers and increased worker mortality (up to an order of magnitude difference) on days 5-6 following treatment compared to colonies in control and chlorantraniliprole-treated plots and clothianidin-exposed colonies also exhibited significantly slower growth (reduced weight by approximately 50% by day 42) over the 6-week post-exposure period. Colonies exposed to the clothianidin-treated turf also did not produce any new queens.

In a separate part of the experiment, bumble bee colonies that had been exposed for two weeks to clothianidin-treated turf containing flowering white clover showed significantly higher worker

and brood mortality (approximately 4x worker mortality and 3x brood mortality compared to controls) and produced fewer honey pots compared to chlorantraniliprole-treated or untreated controls (approximately 35% inhibition compared to controls). Colonies were also introduced into turf 1 week following mowing of the original treated lawn (3 weeks after original treatment). Colonies placed for 14 days on mowed turf that prior to mowing had been treated with either clothianidin or chlorantraniliprole showed no adverse effects compared with control colonies.

Methods

This study was conducted on turf at the A.J. Powell Turf Research Center at the University of Kentucky near Lexington, KY. The turf was Kentucky bluegrass (*Poa pratensis*) with approximately 30% cover (visual estimate) of flowering white clover (*Trifolium ripens*). The first experiment was conducted on a 1-ha sward of this turf. 10 replicate plots (3.35m x 3.35m) were established for each insecticide treatment and control and each replicate was at least 2 meters apart. Treatments were foliar applications of clothianidin (Arena 50[®] WDG, %a.i. not reported, but the only EPA registered label bearing this name is for EPA registration number 59639-152 containing 50% a.i. clothianidin), chlorantraniliprole (Acelepryn[®], 18.4% a.i., this is likely EPA registration number 100-1489) on May 14, 2012 using separate handheld booms for each treatment. Both products were applied for scarab grub control, clothianidin at 0.45 kg a.i./ha (0.401 lbs/acre) and chlorantraniliprole at 0.23 kg a.i./ha (0.205 lbs/acre). About 1 hour after application, the plots were watered in using sprinkling cans.

100 flowers (presumed to be non-pollinated as defined by lacking drooped brown basal florets typical of clover post-pollination) were collected from 5 of the clothianidin-treatment plots on Day 6 after application. Study authors were unable to collect non-pollinated flowers from the chlorantraniliprole and control plots at this time and sampled 100 non-pollinated flowers from 5 distinct untreated areas outside the enclosures. Nectar samples (~300 mg/100 flower samples) were extracted and analyzed for clothianidin residues at the USDA-AMS National Science Laboratory in Gastonia with a level of detection (LOD) of 1.0 ppb using liquid chromatography separation with tandem mass selective detection (LC/MS/MS). The level of quantification (LOQ) for clothianidin residues was not reported in the study.

Commercial bumble bee (*Bombus impatiens*) colonies were placed in screen enclosures erected on each plot two days after the spray application. Hives were randomly assigned to each treatment and control after being blocked by initial weights. Each hive started with 20 workers and a fertilized queen and shipped with a syrup food sack that provided additional food while the colonies were confined in their enclosures. Each enclosure was inspected in the afternoon on the 5th and 6th days post introduction. Workers foraging within the enclosure were counted at these times. In the evening of day 6 after exposure (May 23), hives were closed, weighed and transported 12 km to a 700 ha horse farm in Lexington, KY which the authors reported was free

Commented [WM1]: Label contains language saying not to apply to blooming, pollen-shedding or nectar-producing parts of plants if bees may forage on plants during this time period. For turfgrass specifically, the label says not to apply to plants in bloom if bees are foraging the turf area. Max rate labeled application is 0.4 lbs ai/A. Therefore, for the experimental scenario to make sense for a registered use, bees would have to come in and forage soon after application, but not be present during application.

Commented [WM2]: This is the max usage for white grub (scarab beetle) control. The label does allow for a slightly higher rate (up to 0.26 lbs a.i./A) for other target pests. However, the label also provides a provision for if there are large pest infestation, then it can be applied at up to 0.313 lbs ai/A to control any of the target pests. The label does not say anything regarding avoiding bloom applications.

Commented [WM3]: How does this compare with typical bumble bee colonies in late May/early June?

of any outdoor insecticide applications. Surrounding pastures to the horse farm were also reported to be free of any pesticide applications.

Colonies were allowed to openly forage at the post-exposure site for 6 weeks. Inspections and weight measurements were taken on May 31 and June 13. Colonies were removed to the lab on July 3 and kept at 4.4°C prior to final weights and dissection over the following 1.5 weeks. Colony parameters assessed included numbers of living and dead adults, queens, honey pots and living and dead brood as well as live adult and queen weights.

In a separate part of the study, bumble bee colonies were erected on plots 24 hours after applications (as described above) on June 1, 2012. In this case, some plots were watered in after application, while in others the treatments were allowed to dry on the turf surface and the bumble bee colonies were kept in the enclosures on the plots for two weeks before the hives were brought to the lab. Following removal of this group the sward was mowed to remove clover flowers and new colonies were established one week after mowing when new clover blooms had formed. These colonies were also kept in enclosures on the plots for two weeks before removal to the lab.

In a final part of the study, plots (5 3.7m x 3.7m plots per treatment) were treated as described above with clothianidin, chlorantriliprole or untreated control on May 23, 2012. Untreated borders (2.44 meters) surrounded each plot. These plots were not watered in and no rainfall occurred during the trial. Bee counts were taken in each plot and border every day for one week, counting the number of honey bees (*A. mellifera*) and bumble bees (*B. sp.*) foraging on the clover for 2 minutes over a 45 minute period between 10:30 and 16:00. Bees were freely allowed to forage and therefore may have been counted in multiple plots during the same count.

Statistics

Statistical analyses were performed using Statistix 9 (Analytical Software, 2008). ANOVA tests were used to compare the number of foragers in enclosures, final colony weights and parameters measured during colony dissections. For parameters that were percentages, the study authors used angular transformations and used square root or log transformations for data that did not meet assumptions of equal variance. Non-parametric tests were used for the number of new queens, which did not meet ANOVA assumptions.

Results

The study authors reported that nectar from the clover flower samples collected from clothianidin-treated plots one week after treatment contained 171±44 ppb a.i.. Nectar samples from flowers in non-treated open areas contained no detectable residues of any insecticides.

Flowers that had been under the enclosures in control or chlorantraniliprole-treated plots generally were pollinated which prevented the extraction of sufficient nectar for analysis.

Figure 1: Mean Number of Foraging (1A) and Dead Workers (1B) to Treated Turf During the Exposure Period. (Friedman tests, $p < 0.001$).

Bumble bee colonies in the enclosures on clothianidin-treated turf were reported to have significantly reduced foraging activity and increased worker mortality after 5 and 6 days of exposure (**Figures 1A and 1B**, reproduced from Larson et al, 2013) compared to colonies in enclosures on control and chlorantraniliprole-treated turf. These colonies also showed slower weight gain during the post-exposure phase (**Fig. 2**, reproduced from Larson et al, 2013). The mean weight differences were statistically significant at days 7, 15 and 28 (all $p < 0.01$) but were not statistically significant at day 42 ($p = 0.07$). The study authors reported that there were no statistically significant differences at the time of hive dissection for live adults (workers and drones), honey pots, and colony weight (**Table 1**, reproduced from Larson et al, 2013). Nevertheless, the study authors reported consistent adverse trends (mean inhibitions approximately 36%, 53%, and 17%, respectively, with associated $p = 0.052, 0.09, 0.058$, respectively) for these parameters after six weeks post exposure from the colonies that had been in enclosures on the clothianidin-treated turf compared with the colonies that had been in the control enclosures. During the post-exposure phase, colonies that had previously foraged in enclosures on the clothianidin-treated turf did not produce any new queens. There were no reported significant differences in queen production between colonies that had been in enclosures on control and chlorantraniliprole-treated turf (**Figure 3**, reproduced from Larson et al., 2013)

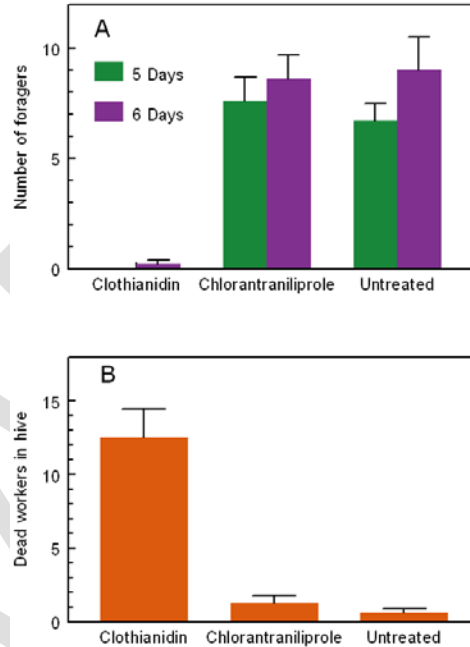


Figure 2: Colony Mean Weight Change Following Exposure to Treated Turf. Mean (\pm SE) weight change (g) of *B. impatiens* colonies during the post exposure phase. (Repeated measures ANOVA: $p < 0.001$ for treatment and date; $p < 0.05$ for treatment \times date interaction). Clothianidin-exposed colonies gained less weight than the other treatments on all dates ($p < 0.01$ at 7, 15, and 28 days after introduction, $p = 0.07$ at 42 days after introduction).

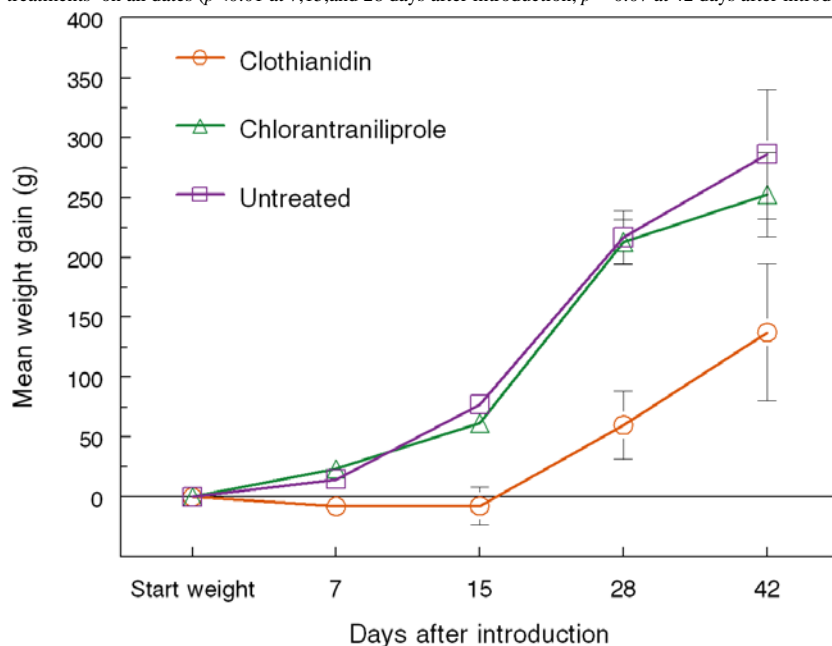


Table 1. Condition of *B. impatiens* colonies that had been exposed to insecticide-treated turf with flowering white clover for 6 days, after which they were moved to an insecticide-free site to openly forage for 6 weeks before this evaluation^a. The turf was lightly irrigated after insecticide application; the surface had thoroughly dried before bees were introduced.

Treatment	Adults (workers and males) per hive			Immatures per hive ^b		Honey Pots	Total wt (g) of live adults	Hive wt (g)
	Live	Dead	% Dead	Live	Dead			
Clothianidin	173 \pm 39	33 \pm 7	31.8 \pm 11.1	84 \pm 15	9 \pm 3	36 \pm 12	28.2 \pm 6.9	709 \pm 59
Chlorantraniliprole	199 \pm 31	35 \pm 14	17.4 \pm 7.3	45 \pm 10	18 \pm 9	51 \pm 10	31.4 \pm 4.8	826 \pm 35
Untreated	271 \pm 30	54 \pm 16	18.2 \pm 5.9	65 \pm 14	27 \pm 13	77 \pm 22	42.9 \pm 5.6	857 \pm 56

^a Data are means (\pm SE). ANOVA (df = 2, 18): live, $F = 2.31$, $P = 0.13$; dead, $F = 0.92$, $P = 0.42$; % dead, $F = 0.93$, $P = 0.41$; wt live adults, $F = 1.8$, $P = 0.19$; live immature, $F = 2.45$, $P = 0.12$; dead immature, $F = 0.90$, $P = 0.42$; honey pots, $F = 2.31$, $P = 0.13$; hive wt., $F = 2.27$, $P = 0.13$. P -values from pre-planned linear contrasts between clothianidin versus untreated were 0.053, 0.23, 0.27, 0.28, 0.20, 0.09, 0.09, and 0.058, respectively. For chlorantraniliprole versus untreated, they were 0.15, 0.29, 0.95, 0.29, 0.51, 0.27, 0.18, and 0.67, respectively.

^b larvae, pupae, and fully-formed workers and males still enclosed in the pupal exoskeleton within the cell.

^c adult workers, males and queens.

Commented [WM4]: Tables did not transfer well from the pdf, so I had to recreate them.

Figure 3: Queen Production Following Exposure to Treated Turf. Mean (+ SE) numbers of queens produced by *B. impatiens* colonies that foraged for 6 days on insecticide-treated lawn turf with white clover and were then moved to an insecticide-free site to openly forage another 6 weeks (Friedman tests: Immature queens, $P = 0.03$; Adult queens, $P = 0.08$; Total queens, $P = 0.05$. Numbers of colonies (out of 10) that produced new queens were 0, 7, and 6 for clothianidin, chlorantraniliprole, and untreated hives, respectively. For the subset of colonies that produced new queens, those exposed to chlorantraniliprole-treated or untreated weedy turf produced similar numbers of immature, adult, and total queens (Kruskal-Wallis test, $P = 0.69, 0.84, 0.95$, respectively). Queens present in clothianidin exposed colonies likely represent the original mother queen.

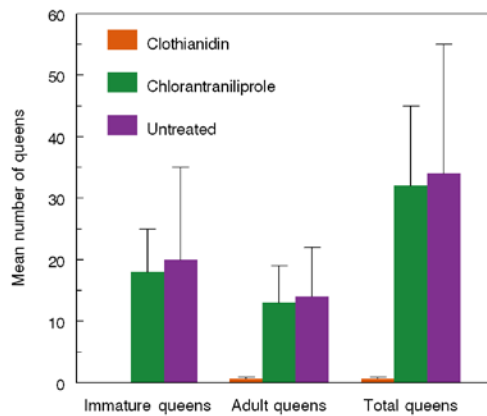


Table 2 (reproduced from Larson *et al.*, 2013) summarized the colony assessment of bumble bee colonies that were exposed in the enclosures on unmowed treated and untreated turf for two weeks. Bumble bee colonies that had been in enclosures on clothianidin-treated turf exhibited significantly increased worker and brood mortality (~3.7x and ~3.3x control mortality, respectively) and produced significantly fewer honey pots (~35% inhibition) compared to colonies in enclosures on untreated turf. Bumble bee colonies that had been exposed on the chlorantraniliprole-treated plots showed no significant differences compared with colonies from the untreated turf. **Table 3** (summarized from Larson *et al.*, 2013) summarized the colony assessment for bumble bee colonies that were exposed in the enclosures on turf that had been mowed after treatment, but prior to hive installation and had developed new flowers. There were no significant differences between colonies that had been on enclosures in the clothianidin, chlorantraniliprole, or control turf plots in this scenario, except that hives that had been on the chlorantraniliprole-treated turf had significantly higher numbers of live adult workers than the untreated controls (two-tailed Dunnett's test, $p = 0.02$). There were no immature live bees in the colonies that had been in enclosures on the untreated turf after mowing.

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Table 2: Condition of *B. impatiens* colonies that were evaluated after being exposed to insecticide-treated or untreated turf with flowering white clover for two weeks. Plots treated June 1, 2012; colonies introduced June 2. Data are means (\pm SE). ANOVA (df = 2,22): live, $F=4.57$, $P<0.05$; dead, $F=9.88$, $P<0.01$; wt live workers, $F=3.46$, $P=0.05$; live immature, $F=0.57$, $P=0.57$; dead immature, $F=9.25$, $P<0.01$; honey pots, $F=3.56$, $P<0.05$; hive wt, $F=0.69$, $P=0.51$.

	Adult Workers per Hive ^a		Immatures per hive ^b		Honey Pots	Total Weights (g) of live adults ^c	Hive Weight (g)
	Live	Dead	Live	Dead			
Clothianidin	59 \pm 12*	26 \pm 5*	21 \pm 8	13 \pm 2*	33 \pm 5*	7.7 \pm 1.4*	580 \pm 17
Chlorantriliprole	99 \pm 12	6 \pm 2	31 \pm 9	4 \pm 1	47 \pm 5	12.2 \pm 1.5	599 \pm 11
Untreated	106 \pm 8	7 \pm 3	17 \pm 10	4 \pm 1	51 \pm 4	12.8 \pm 1.6	602 \pm 6

* denotes means significantly higher or lower than colonies on untreated turf (Dunnett's test, $\alpha=0.05$)

^aAll adults (other than original queen) were workers as there would not have been time for males to emerge from the brood.

^blarvae, pupae, and fully-formed workers still enclosed in the pupal exoskeleton within the cell.

^cadult workers and original queen

Table 3: Condition of *B. impatiens* colonies that were evaluated after 2 weeks exposure to turf with flowering white clover that had bloomed after the sward was mown to remove flowers present at the time of treatment. Insecticide application, mowing, and introduction of bee colonies were on June 1, 15, and 22, respectively. Data are means (\pm SE). ANOVA (df=2,19): live, $F=6.01$, $P=0.02$; dead, $F=1.05$, $P=0.37$; wt live workers, $F=3.31$, $P=0.08$; live immature, $F=1.60$, $P=0.25$; dead immature, $F=0.54$, $P=0.6$; honey pots, $F=2.15$, $P=0.17$; hive wt, $F=1.93$, $P=0.20$.

	Adult Workers per Hive ^a		Immatures per hive ^b		Honey Pots	Total Weights (g) of live adults ^c	Hive Weight (g)
	Live	Dead	Live	Dead			
Clothianidin	93 \pm 9	11 \pm 4	12 \pm 8	6 \pm 1	52 \pm 6	13.0 \pm 1.3	585 \pm 11
Chlorantriliprole	130 \pm 12*	7 \pm 2	8 \pm 4	6 \pm 2	69 \pm 6	16.7 \pm 1.6	621 \pm 16
Untreated	81 \pm 8	7 \pm 2	0	3 \pm 1	56 \pm 3	11.3 \pm 0.9	588 \pm 8

*Significantly higher than untreated; 2-tailed Dunnett's test.

^aAll adults (other than original queen) were workers as there would not have been time for males to emerge from the brood.

^blarvae, pupae, and fully-formed workers still enclosed in the pupal exoskeleton within the cell.

^cadult workers and original queen.

No significant differences were observed in the number of bumble and/or honey bees foraging in the clothianidin-treated, chlorantraniliprole-treated or control areas.

Data Quality Evaluation

No standard performance criteria of bumble bee colonies in control conditions has currently been identified by EPA or PMRA; therefore the performance exhibited by control colonies in this experiment cannot be assessed. However, some bumble bee cultivation literature (Evans *et al.*, 2007) indicates a range of peak colony worker numbers (200-500) that encompasses the control

colony numbers in this study after six weeks of growth. The study authors started their colonies with a queen and 20 workers, which is the minimum strength that Evans *et al.*, recommends before allowing workers to openly forage.

Similarly, no existing guidelines are available for field tests conducted with *Bombus impatiens*. Replication, while not large (n=10 plots/treatment) was sufficient to statistically differentiate between colonies in clothianidin-treated areas and colonies in control or chlorantriliprole-treated areas. The results of the study appeared to corroborate the results of laboratory neonicotinoid sub-lethal exposures in bees when concentrations are relatively high, though below lethal (*i.e.* LD₅₀) concentrations.

The residue data on concentrations of clothianidin in clover nectar following foliar applications of clothianidin to turf under the study conditions provide useful information. Although the study authors do not provide the LOQ for the study, concentrations measured were well over the LOD and are likely of sufficient quality for use in risk assessment. The study authors did not report clothianidin concentrations in nectar of clover plants following mowing, which might have provided valuable information regarding the systemic movement of clothianidin in clover following foliar applications as well as providing corresponding residue levels that do not result in measurable effects to bumble bee colonies.

10. Peer Review

Primary Reviewer Comments

Rationale for Use:

This study provides information on residues of clothianidin following foliar application on turf when clover is flowering. This study provides information that contributes to the understanding of potential clothianidin exposures and effects to bumble bee colonies when bees are actively foraging shortly after application at the maximum rates registered for clothianidin. Exposures are sub-lethal (*i.e.* below the LD₅₀), but it is not clear how typical of environmentally relevant conditions the tested situation would appear to be, given the label language designed to prevent applications to blooming plants or when bees are actively foraging.

Limitations of Study:

Clothianidin is labeled for use on turfgrass at the application rates used in this study (which is the maximum application rate currently registered for all clothianidin use sites). However, current labels include language stating not to apply to blooming, pollen-shedding or nectar-producing parts of plants if bees may forage on plants during application and for at least 5 days following treatment. The study authors state that “applications are sometimes made when lawn weeds such as dandelions and white clover are flowering.” However, such applications would seem to be a

misuse of clothianidin and therefore it is uncertain whether the tested scenario represents typical environmental conditions. The concentrations observed in clover nectar in this study were considerably higher than concentrations generally reported in the literature or registrant submitted studies for other use patterns. Therefore, there is some uncertainty as to the utility of the effects data presented except where foliar applications are permitted and bees forage at or shortly after the time of application.

Both pollen and nectar food sources may be contaminated with neonicotinoids, whereas this study only considered the nectar exposure route. If pollen is also contaminated with neonicotinoid residue levels similar to those observed in this study, then there may be potential for additional effects. Conversely, bees in the enclosures in this study had no choice but to consume nectar containing clothianidin residues, whereas in actual field conditions, the diet may be more diverse and contain food sources without neonicotinoids.

Adverse effects were not observed when colonies were placed *in situ* following mowing (3 weeks after treatment), however residues were not measured in clover blooms that came up following mowing, which to some extent limits the value of this data.

For chlorantraniliprole, maximum labeled use rates are somewhat higher (0.26 lbs a.i./A for other target pests or 0.313 lbs a.i./A, when pest infestations are high) than the tested rates. The results for chlorantraniliprole effects on bumble bee colonies are useful under typical use rates for turfgrass' target pest from this study (scarab/white beetle grubs), however this may underestimate actual effects when maximum use rates are used.

Description of Use in Document:

Qualitative. The residue data may be used qualitatively to represent residues immediately following foliar application at the maximum application rate for clothianidin. The study may also be used in risk assessment to characterize the effects of sub-lethal, but very high clothianidin concentrations on bumble bee colonies, which may be useful for comparison where foliar applications occur and bees are present or forage on the applied area shortly after treatment or where empirical clothianidin residue data is not available and modeled concentrations are in the range suggested by this study.

Secondary Reviewer Comments:

[Provide any comments from secondary reviewer. Comments should be high level (e.g., related to the conclusions of the study, major flaws in design, or how it is used in risk assessment)]

Resolution:

[Provide a description of the resolution if there is a discrepancy between the primary and the secondary reviewer]

11. References:

Evans, E., I. Burns, and M. Spivak. 2007. Befriending Bumble Bees: A Practical Guide to Raising Local Bumble Bees. University of Minnesota Extension.

Primary Reviewer (EPA): Michael Wagman, Biologist
Environmental Risk Branch VI
Environmental Fate and Effects Division

Date: 11/27/14

Secondary Reviewer (PMRA):

Date: